United States
Environmental Protection
Agency

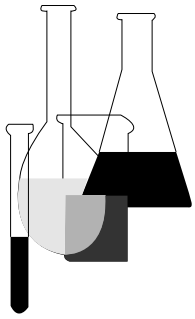
Prevention, Pesticides
and Toxic Substances
(7101)

EPA 712–C–96–255
June 1996

EPA

Health Effects Test Guidelines

OPPTS 870.8360 Pharmacokinetics of Isopropanal



“Public Draft”

INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines’’ or in paper by contacting the OPP Public Docket at (703) 305–5805 or by e-mail: guidelines@epamail.epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: guidelines@epamail.epa.gov.

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on *The Federal Bulletin Board*. By modem dial 202–512–1387, telnet and ftp: fedbbs.access.gpo.gov (IP 162.140.64.19), or call 202–512–0132 for disks or paper copies. This guideline is also available electronically in ASCII and PDF (portable document format) from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines.’’

OPPTS 870.8360 Pharmacokinetics of isopropanal.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline is OPPT 40 CFR 795.231 Pharmacokinetics of Isopropanal.

(b) **Purpose.** The purpose of these studies is to ascertain whether the pharmacokinetics and metabolism of the test substance are similar after oral and inhalation administration; determine bioavailability of the test substance after oral and inhalation administration; and to examine the effects of repeated dosing on the pharmacokinetics and metabolism of the test substance.

(c) **Definitions.** The definitions in section 3 of TSCA and in 40 CFR Part 792—Good Laboratory Practice Standards (GLP) apply to this test guideline. The following definitions also apply to this test guideline.

Bioavailability refers to the rate and relative amount of administered test substance which reaches the systemic circulation.

Metabolism means the study of the sum of the processes by which a particular substance is handled in the body, and includes absorption, tissue distribution, biotransformation, and excretion.

Pharmacokinetics means the study of the rates of absorption, tissue distribution, biotransformation, and excretion.

(d) **Test procedures**—(1) **Animal selection**—(i) **Species.** The rat should be used because it has been used extensively for metabolic and toxicological studies.

(ii) **Test animals.** For pharmacokinetics testing, adult male and female rats (Fischer 344 or strain used for major toxicity testing), 7 to 9 weeks of age, should be used. The animals should be purchased from a reputable dealer and should be identified upon arrival at the testing laboratory. The animals should be selected at random for the testing groups and any animal showing signs of ill health should not be used. In all studies, unless otherwise specified, each test group should contain at least four animals of each sex for a total of at least eight animals.

(iii) **Animal care.** (A) Animal care and housing should be in accordance with DHEW Publication No. (NIH)–85–23 (1985), Guidelines for the Care and Use of Laboratory Animals.

(B) The animals should be housed in environmentally controlled rooms with at least 10 air changes per hour. The rooms should be main-

tained at a temperature of 22 ± 2 °C and humidity of 50 ± 20 percent with a 12–h light/dark cycle per day. The animals should be kept in a quarantine facility for at least 7 days prior to use and should be acclimated to the experimental environment for a minimum of 48 h prior to treatment.

(C) During the acclimatization period, the animals should be housed in suitable cages. All animals should be provided with certified feed and tap water ad libitum.

(2) **Administration of test substance**—(i) **Test substance.** The use of radioactive test substance is required for all materials balance and metabolite identification requirements of the study. The purity of both radioactive and nonradioactive test substance should be greater than 99 percent. The radioactive and nonradioactive substances should be chromatographed separately and together to establish purity and identity. If the purity is less than 99 percent or if the chromatograms differ significantly, EPA should be consulted.

(ii) **Dosage and treatment**—(A) **Intravenous.** The low dose of test substance, in an appropriate vehicle, should be administered intravenously to four rats of each sex.

(B) **Oral.** Two doses of test substance should be used in the oral portion of the study, a low dose and a high dose. The high dose should induce some overt toxicity, such as weight loss. The low dose level should correspond to a no-observed-effect level. The oral dosing should be accomplished by gavage or by administering an encapsulated test substance. If feasible, the same high and low doses should be used for oral and dermal studies.

(C) **Inhalation.** Two concentrations of the test substance should be used in this portion of the study, a low concentration and a high concentration. The high concentration should induce some overt toxicity, while the low concentration should correspond to a no-observed-effect level. Inhalation treatment should be conducted using a nose-cone or head-only apparatus to prevent ingestion of the test substance through grooming.

(iii) **Dosing and sampling schedule.** After administration of the test substance, each rat should be placed in a separate metabolic unit to facilitate collection of excreta. For the inhalation studies, excreta from the rats should also be collected during the exposure periods. At the end of each collection period, the metabolic units should be cleaned to recover any excreta that might adhere to the cages. All studies, except the repeated dose study, should be terminated at 7 days, or after at least 90 percent of the radioactivity has been recovered in the excreta, whichever occurs first.

(A) **Intravenous study.** Group A should be dosed once intravenously at the low dose of test substance.

(B) **Oral studies.** (*1*) Group B should be dosed once orally with the low dose of the test substance.

(*2*) Group C should be dosed once orally with the high dose of the test substance.

(C) **Inhalation studies.** A single 6–h exposure period should be used for each group.

(*1*) Group D should be exposed to a mixture of the test substance in air at the low concentration.

(*2*) Group E should be exposed to a mixture of test substance in air at the high concentration.

(D) **Repeated dosing study.** Group F should receive a series of single daily oral low doses of nonradioactive test substance over a period of at least 7 consecutive days. A single oral low dose of radioactive test substance should be administered 24 h after the last nonradioactive dose. Following dosing with radioactive substance, the rats should be placed in individual metabolic units as described in paragraph (d)(2)(iii) of this guideline. The study should be terminated 7 days after the last dose, or after at least 90 percent of the radioactivity has been recovered in the excreta, whichever occurs first.

(3) **Types of studies**—(i) **Pharmacokinetics studies.** Groups A through F should be used to determine the kinetics of absorption of the test substance. In groups administered the substance by intravenous or oral routes, (i.e., Groups A, B, C, F), the concentration of radioactivity in blood and excreta including expired air should be measured following administration. In groups administered the substance by the inhalation route (i.e., Groups D and E), the concentration of radioactivity in blood should be measured at selected time intervals during and following the exposure period. In the groups administered the substance by inhalation (i.e., Groups D and E), the concentration of radioactivity in excreta (including expired air) should be measured at selected time intervals following the exposure period. In addition, in the groups administered the substance by inhalation, the concentration of test substance in inspired air should be measured at selected time intervals during the exposure period.

(ii) **Metabolism studies.** Groups A through F should be used to determine the metabolism of the test substance. Excreta (urine, feces, and expired air) should be collected for identification and quantification of test substance and metabolites.

(4) **Measurements—pharmacokinetics.** Four animals from each group should be used for these measurements.

(i) **Bioavailability.** The levels of radioactivity should be determined in whole blood, blood plasma or blood serum at 15 min, 30 min, 1, 2,

3, 6, 9, and 18 h after dosing; and at 30 min, 3, 6, 6.5, 7, 8, 9, 12, and 18 h after initation of inhalation exposure.

(ii) **Extent of absorption.** The total quantities of radioactivity should be determined for excreta collected daily for 7 days, or after at least 90 percent of the radioactivity has been recovered in the excreta, whichever occurs first.

(iii) **Excretion.** The quantities of radioactivity eliminated in the urine, feces, and expired air should be determined separately at appropriate time intervals. The collection of the intact test substance or its metabolites, including carbon dioxide, may be discontinued when less than 1 percent of the administered dose is found to be exhaled as radioactive carbon dioxide in 24 h.

(iv) **Tissue distribution.** At the termination of each study, the quantities of radioactivity in blood and in various tissues, including bone, brain, fat, gastrointestinal tract, gonads, heart, kidney, liver, lungs, muscle, skin, spleen, and residual carcass of each animal should be determined.

(v) **Changes in pharmacokinetics.** Results of pharmacokinetics measurements (i.e., biotransformation, extent of absorption, tissue distribution, and excretion) obtained in rats receiving the single low oral dose of test substance (Group B) should be compared to the corresponding results obtained in rats receiving repeated oral doses of test substance (Group F).

(vi) **Biotransformation.** Appropriate qualitative and quantitative methods should be used to assay urine, feces, and expired air collected from rats. Efforts should be made to identify any metabolite which comprises 5 percent or more of the dose eliminated.

(vii) **Changes in biotransformation.** Appropriate qualitative and quantitative assay methodology should be used to compare the composition of radioactive substances in excreta from the rats receiving a single oral dose (Groups B and C) with those in the excreta from rats receiving repeated oral doses (Group F).

(e) **Data and reporting.** The final test report should include the following:

(1) **Presentation of results.** Numerical data should be presented in tabular form. Pharmacokinetics data should also be presented in graphical form. Qualitative observations should be reported.

(2) **Evaluation of results.** All quantitative results should be evaluated by an appropriate statistical method.

(3) **Reporting results.** In addition to the reporting requirements as specified in 40 CFR 792.185, the following specific information should be reported:

(i) Species and strains of laboratory animals.

(ii) Chemical characterization of the test substance, including:

(A) For the radioactive test substance, information on the sites and degree of radiolabeling, including type of label, specific activity, chemical purity, and radiochemical purity.

(B) For the nonradioactive substance, information on chemical purity.

(C) Results of chromatography.

(iii) A full description of the sensitivity, precision, and accuracy of all procedures used to generate the data.

(iv) Extent of absorption of the test substance as indicated by percent absorption of the administered oral dose and total body burden after inhalation exposure.

(v) Quantity and percent recovery of radioactivity in feces, urine, expired air, and blood.

(vi) Tissue distribution reported as quantity of radioactivity in blood and in various tissues, including bone, brain, fat, gastrointestinal tract, gonads, heart, kidney, liver, lung, muscle, skin, spleen and in residual carcass of each rat.

(vii) Biotransformation pathways and quantities of the test substance and metabolites in excreta collected after administering single high and low doses to rats.

(viii) Biotransformation pathways and quantities of the test substance and metabolites in excreta collected after administering repeated low doses to rats.

(ix) Pharmacokinetics models developed from the experimental data.